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Quantitative Analysis of MDR-1 Gene Expression in Acute Myeloid Leukemia by Reverse Transcription Polymerase Chain Reaction

To the Editor: Drug resistance is the main cause of treatment failure of acute myeloid leukemia (AML). Expression of the multidrug resistance (MDR) protein, P-glycoprotein 170 (P-gp), encoded by the MDR-1 gene, has been suggested as playing an important role in this mechanism. It was identified that the cancer cells acquired the capacity of increasing drug efflux through activating on energy-dependent membrance transport pump whose molecular basis is P-gp [1]. We used the reverse transcription polymerse chain reaction (RT-PCR) technique to investigate P-gp expression in AML. Twenty patients (female 10, male 10) with newly diagnosed AML were included in our study. Ages ranged from 20 to 82 years, with a median of 50 years, and the bone marrow (BM) aspirates were taken for cytological classification and AML phenotype according to the French-American-British (FAB) criteria. The proportion of leukemia blasts in their BM varied between 30% and 100%, with a median of 96%. After diagnosis, the chemotherapy was given on the protocol of the German AML Co-operative Group [2]. Twelve patients achieved subsequently complete remission (CR). The overall CR rate was 66%. The controls were provided by normal peripheral blood leukocytes from healthy volunteers, and by an MDR-negative Chinese hamster ovarian cell line and its colchicum-selected MDR variant (CHO-RT).

Total RNA (tRNA) was extracted with a guanidinium isothiocyanate buffer by phenol–chloroform and assessed spectrophotomertically. A five-carat cell cDNA was synthesized from 5 μg of tRNA using 100 ng of random hexadeoxynucleotide primer in 30 μl of a reaction solution at 37°C for 1 hr. PCR was performed with cDNA derived from 80 ng of RNA and reaction kits in a final volume of 25 μl. PCR conditions were: 94°C 45 secs, 60°C 1 min, 72°C 2 min, for a total of 40 cycles. *MDR-1* gene-specific sequences were amplified by using the sense-strand primer CCCATCATTG-CAATAGCAGG (residues 2596–2615) and the antisense-strand primer GTTCAAACTTCTGCT-CCTGA (residues 2733–2752), added at 37.5 pmol per reaction. PCR products were separated on 1.5% agarose gels and visualised by ultraviolet (UV) illumination. Several negative control reactions that contained water or cDNA reaction mixtures without RNA were included in each experiment.

Eighteen samples expressed *MDR-1* gene product, while other two suffering from biphenotypic AML were negative. The total *MDR-1* gene expression rate was 90%. P-gp expression did not correlate with age, sex, or FAB classification. By statistical analysis, the CR rate was not significantly different between patients with *MDR-1* gene expression and those without detectable expression. Eight of 8 patients without CR were *MDR-1* gene positive, while 10 of 12 patients with CR were positive as well. No significant correlation of *MDR-1* gene expression to other cell surface markers (CD2, CD13, CD14, CD19, CD33, TdT) was found. However, 7 of 8 nonresponders were positive for CD34, whereas 4 of 12 responders were positive (data not shown).

P-gp excludes drugs from the interior of cells to protect lymphocyte from toxic body product. Its distribution was thought to serve as a chloride ion channel, ATP channel, a membrane ATPase [1]. A recent report suggested that they may block drug or dye transport by efficiently competing for binding site on the transport pump or its cancer protein [3]. Our study demonstrated a fairly high incidence of *MDR-1* gene expression in AML, even those who never received any chemotherapy. However, no difference in the incidence of *MDR-1* gene expression between newly diagnosed patients and relapsed patients was found, as well no linear relationship between *MDR-1* gene expression and achievement of CR in patients with newly diagnosed AML or relapse. Hence, we cannot confirm that *MDR-1* gene expression could be used to predict the outcome of treatment of patients with AML [4]. The same observation has also been reported [5]. Interestingly, we found a higher level of CD34 to be a negative prognostic factor independent of *MDR-1* gene expression in AML. Therefore, many other molecular biological works, such as multidrug resistance associate protein (MRP), topoisomerase I and II, and glutathione, remain to be done before any firm conclusions can be drawn [4].

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Refractory Pancreatitis Associated With Graft-Versus-Host Disease in Fanconi Anemia

To the Editor: Fanconi anemia (FA) is an autosomal recessive disorder characterized by a high incidence of aplastic anemia, congenital malformations, and a constitutional chromosomal fragility syndrome [1]. Although the outcome of bone marrow transplantation (BMT) in FA patients has been improved by the use of decreased doses of cyclophosphamide (CY) as a preconditioning regimen, acute graft-versus-host disease (GVHD) is still the main cause of death after BMT in FA patients [2,3]. We present the susceptibility of the anomalous pancreas to GVHD in a FA patient after allogeneic BMT.

A 4-year-old girl with pancytopenia was referred to our hospital in 1993. Past history revealed only paleness of the patient over the previous years. Family history revealed two healthy siblings and healthy parents. Physical examination revealed short stature, microcephaly, skin pigmentation, and mental retardation. The remainder of the physical examination was normal.

There were no signs of a pancreatic anomaly on radiographs. Laboratory findings were as follows: Hb 5.0 g/dl, WBC 3.3 × 10^9/L, platelets 34 × 10^9/L and HbF 14.1%. The serum amylase level was normal. Bone marrow aspiration showed hypocellular marrow, fewer than 1% blasts, and dysplastic features in erythroid, granulocytic, and megakaryocytic lineages. The bone marrow karyotype was normal. The diagnosis of FA associated with myelodysplastic syndrome (MDS) was made based on clinical characteristics and further supported by chromosome breakage in peripheral lymphocytes following treatment with mitomycin C. In 1994, allogeneic BMT from the patient's HLA-identical male sibling was performed using low-dose CY (5mg/kg/day × 4) followed by 5-GY total body irradiation as the conditioning regimen. The patient received cyclosporin A (3 mg/kg/day), methotrexate (10 mg/m^2 on day 1; 6 mg/m^2 on days 3, 6, and 11) and prednisolone (0.5 mg/kg/day) for GVHD prophylaxis. Although successful engraftment was confirmed by sex chromosome karyotyping on day 13, the patient had grade III GVHD beginning on day 15 after BMT, with skin, liver, and gut manifestations. Her clinical evolution was further complicated on day 19 by severe pancreatitis of unknown origin. The clinical signs of skin and gastrointestinal GVHD improved to some degree, but pancreatitis was refractory to intensive chemotherapy.

The patient died on day 98 post-graft due to severe pancreatitis. Autopsy showed an acute pancreatitis, with acute GVHD compatible infiltration of lymphocytes, and further revealed a congenital hypoplasia of the pancreas with diffuse fibrosis. Thus, the pancreatitis in this patient might have resulted from acute GVHD induced by a anomalous pancreas. Congenital anomaly of the pancreas and the susceptibility of the pancreas to GVHD have not been fully recognized in FA patients. We must now consider the possibility of pancreatitis associated with GVHD in FA patients after allogeneic BMT.

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Central Nervous System Involvement in Multiple Myeloma

To the Editor: Multiple myeloma (MM) commonly presents with neurologic symptoms when it involves the cranium or vertebrae, but rarely invades the central nervous system (CNS) or meninges. Intracranial and intraspinal myeloma without lesions in the adjacent bone are extremely rare [1].

Between 1975 and 1996, 192 patients (108 men, 84 women, median age 56 years) with MM have been observed. Thirty-two patients (16%) had neurologic findings due to various causes. Of these, three patients had a direct involvement of the CNS. First patient, a 42-year-old man was admitted with headache, nausea, vomiting, and seizures. Computed tomography (CT) showed a tumoral mass in the frontal lobe. He had anemia, a high erythrocyte sedimentation rate, and hyperglobulinemia. Serum immonoelectrophoresis confirmed the presence of a monoclonal IgG protein. A bone marrow examination revealed an increase of plasma cells. The mass was removed surgically. The pathologic examination was consistent with plasmacytoma.

The second patient was a 54-year-old woman who had been followed with IgG myeloma. In the third year of the disease, she presented with weakness and wasting in the hands and arms, loss of arm reflexes, and spastic weakness of the lower extremities. A CT scan showed a cervical intraspinal tumoral mass. Peripheral blood smear revealed the presence of plasma cells. She was in poor general condition and died of sepsis within 1 week. On postmortem examination, the mass proved to be an intraspinal plasmacytoma.

The third patient was a 64-year-old man who was admitted to the hospital after he had had a grand mal convulsion. On a CT scan, left parietal lobe tumoral mass was diagnosed. After investigations, IgA myeloma was diagnosed. Pathologic examination of the mass showed sheets of plasma cells.

Nervous system involvement is frequently seen in patients with MM. The most common manifestations are polyneuropathy and myelopathy secondary to spinal cord compression [2]. Polyneuropathy due to autoimmune mechanisms occurs in 5% of patients with MM [3]. Other less common causes of neurologic dysfunction are myelomatous meningitis, amyloidosis, and sensorimotor polyneuropathy due to a remote effect of plasma cells.

Here we present three MM patients with intraparenchymal plasmacytomas without bone or dural attachment. Intracranial involvement with myeloma occurs in one of three forms: single or multiple cranial nerve palsies due to myelomatous involvement of the base of the skull, intraorbital tumors, and intracranial plasmacytoma [4]. The tumor may be the first sign or may develop during the course of the disease. Although it is a less common finding, when a neurologic symptom is discovered in a patient with MM, intraparenchymal CNS plasmacytoma should be considered in the differential diagnosis.

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When Is Selection Bias Not Selection Bias?

To the Editor: Reports from this institution [1] and others [2,3] demonstrated that clonal cytogenetic abnormalities can be used as prognostic factors for adults with newly diagnosed acute myelogenous leukemia (AML). With conventional chemotherapy, those with "good prognosis" karyotypes (t(15;17), t(8;21) and inv16) have a 30–50% long-term disease-free survival,